

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

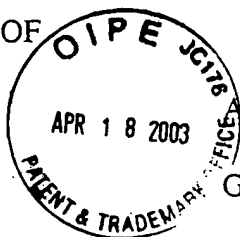
In re PATENT APPLICATION OF

*GUICHARD et al.*

Serial No.: 08/716,249

Filed: September 13, 1996

For: RETRO PEPTIDES, ANTIBODIES THERETO AND  
THEIR USES FOR VACCINATION AND IN VITRO  
DIAGNOSIS



Atty. Ref.: 1487-24

Group Art Unit: 1648

Examiner: Parkin, J.

\* \* \* \* \*

132 DECLARATION

Assistant Commissioner of Patents  
Washington, DC 20231

Sir:

We, Sylviane MULLER and Jean-Paul BRIAND, declare as follows.

1. We are French citizens and reside at 15, Avenue de la Farêt Noire, 6700 Strasbourg, France, and 22 rue des Balayeurs, 6700 Strasbourg, France, respectively.
2. We are two of the co-inventors of the claims of the above-identified patent application.
3. Attached are copies of our individual Curriculum Vitae.
4. We have reviewed the above-identified application, including the pending and proposed amendments to the claims, and the related applications PCT/FR 95/00292 and FR 94-02950.

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5. We have reviewed the Office Action mailed November 10, 1998 in the above.

6. On pages 1-4 of the Office Action of November 10, 1998, the Examiner has criticized the adequacy of the above-identified disclosure to teach one of ordinary skill in the art to make and/or use the claimed invention.

7. We believe the above-identified application teaches one of ordinary skill to make and use the claimed invention, as of the time of our invention, and submit the attached documents and the following comments in support of our belief. While we believe many of the Examiner's enumerated comments are repetitive, we attempt to address each in the following. As an overall comment, we note the Examiner refers in many instances to what the "prior art teaches" without supplying copies of such prior art. We supply the following factually supported discussion of the teachings of the art to advance the examination of the above-identified application.

8. The Examiner asserts that

"It is well documented that chemical modifications to peptides often affect the biological activities of such peptides in an unpredictable manner." See, ¶1) on page 2 of Paper No. 26.

We believe this observation is irrelevant to whether one of ordinary skill in the art is taught how to make and use the presently claimed invention. That is, whether the "biological activities" of the parent peptide is "affected" as compared to retro- or retro-inverso peptides made as presently described is only relevant as to whether the antigenic and/or immunogenic activity of the parent peptide is "affected". Even if "affected" to some degree, for better or worse, we believe one of ordinary skill was taught by our specification, and the generally high degree of knowledge in the art, to make and use our claimed invention.

9. We, along with our co-inventors, have demonstrated by specific examples disclosed in the present application that retro- and retro-inverso modifications to antigenic and/or immunoreactive peptides do not "affect" the antigenic and/or immunoreactive properties of the parent peptide to a significant degree. In fact, we believe we have demonstrated that our retro- and retro-inverso peptides mimic the L-peptide with generally improved recognition by antibodies raised against the parent L-peptides, as compared to the parent L-peptide. We have provided four examples of our invention in the above-identified specification. These four examples (see, pages 49-52 of the specification) include retro- and retro-inverso peptides of the following parent peptides.

C-terminal peptide, amino acids 130-135, of histone H3;

internal domain, amino acids 277-291, of the 52 kD SSA/Ro (Ro 52) protein;

internal domain, amino acids 304-324, of the 60 kD SSA/Ro (Ro 60) protein; and

internal domain, amino acids 28-45, of histone H3.

10. The two other examples of our invention are described in the attached publication, S. Muller et al., Peptide Research, Vol. 8, No. 3 (1995), 138-144, (publication number (1)). In this work, we, with others, have synthesized end group-modified retro-inverso analogues of two synthetic peptides corresponding to two FMDV (Foot-and-Mouth Disease Virus) variants of serotype A, subtype 12, which differ only at position 153, FP- and FL-peptides corresponding to the VP1 region 141-159 of the two said FMDV variants. Table 2, p. 140 of this publication shows the amino acid sequences of the parent peptides and retro-inverso analogues of the two FMDV variants (see, also figure 1, p. 140). Figures 1A and 1B, show that antibodies induced



against FP-L- (Pro at amino acids 154) and FL-L- (Leu at amino acids 154) peptides cross-reacted with related RIa and RIb peptides, although, in two out of three rabbits, with a slightly lower titer. Moreover, as shown in Figures 1C-1F, the RIa and RIb peptides could be used to produce antisera which cross reacted with the L-peptides. The authors concluded that

“when compared to the antibody response raised against L-peptides, the duration of the IgG response that was induced by retro-inverso peptides was significantly longer and the titer of anti-peptide antisera was much higher.”

11. The Examiner asserts that

“The disclosure fails to adequately teach which chemical modification will result in the production of retro-inverso peptides or retro peptides with improved half-lives.” See, ¶(2), page 2 of Paper No. 26.

While we do not believe the improved half-life of a retro- or retro-inverso peptide, according to the invention needs to be demonstrated, we note that the half life of the peptides of the invention is implicitly disclosed in figure 3 and the discussion of same on page 27 of the present application which relates to the resistance to trypsin. A more detailed review of this affect of retro- and retro-inverso peptides is provided in the attached publication (2), Briand et al., Proc. Natl. Acad. Sci. USA, Vol. 94, pp. 12545-12550, November 1997, Immunology. Figure 2, p. 12549 of this publication shows studies of the resistance of the parent L-peptide Ac-(C)141-159-OH (i.e., peptide sequence of amino acids 141-159 of the FMDV VP1) and of the retro-inverso analogue NH<sub>2</sub> -(C) 141-159 -OH to trypsin. The half-life of the retro-inverso peptide NH<sub>2</sub> -(C) 141-159 -OH was found to be more than 20-fold greater than that of the L-peptide; i.e., 450 min for the retro-inverso peptide compared with 20 min for the parent peptide. The demonstrated improved half-life of the retro and retro-inverso peptides of the present



invention is a desirable yet not required outcome of the retro- and retro-inverso peptides of the present invention.

12. The Examiner asserts that

“the prior art teaches that retro-inverso peptides or retro peptides often fail to display the requisite biological, immunological, or biochemical activities of the parent peptide.” See, ¶(3), page 2 of Paper No. 26.

13. We do not believe one of ordinary skill in the art would separately require that the “biological”, “immunological” and “biochemical activities” of the retro- and retro-inverso peptides of the present invention be the same as the parent peptide to be able to make and use the claimed invention, as apparently required by the Examiner. The present invention provides retro- and retro-inverso peptides which retain antigenic and/or immunogenic activities which are similar, if not the same as, the parent peptides. The present peptides are known to be antigenic and/or immunogenic. One of ordinary skill would have been able to make and use retro- and retro-inverso forms of the present invention at the time of our invention. The Examiner has not provided or cited to any document which supports his assertion with regard to the alleged “often” loss of immunological activity with retro-inverso or retro modification. The “biological” and “biochemical” activity of retro-inverso or retro-peptide is not the subject of the present invention. The field of our invention is related to the immunological and/or antigenic properties of peptide analogues.

14. The Examiner asserts that

"The prior art teaches that limited chemical modifications or single amino acid additions, deletions or substitutions are sufficient to abrogate antigen-antibody binding interactions",

and, that

"The disclosure fails to adequately teach which retro-inverso peptide-, retro peptide-, or enantiomeric parent peptide-specific antibodies can reasonably be expected to recognize and bind to parent peptides or proteins". See ¶¶4) and 5), pages 2-3 of Paper No. 26.

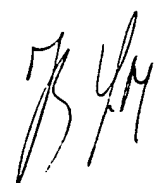
15. These statements must be contested. Several examples of the allegedly missing information are described in the present application. In addition, we note follow and attached. Mezière et al., The Journal of Immunology, 1997, 159 : 3230-3237, attached as document (3) provides the following peptides: peptide 103-115 of poliovirus VP1, which is involved in the production of antibodies that neutralize the infectivity of the virus, and, peptide 435-446 from the third constant region of mouse heavy chain IgG2<sub>a</sub> allopeptide  $\gamma 2a^b$  which mimics a corneal antigen implicated in autoimmune keratitis. The amino acid sequences of parent (blocked) peptides 435-446 of the  $\gamma 2a^b$  allotype and 103-115 of poliovirus type 1 VP1 and of their retro-inverso analogues are given in table 1, page 3231 of the attached document (3). Document (3) details the induction of antibodies to retro-inverso peptide 435-446 of  $\gamma 2a^b$  and peptide 103-115 of poliovirus VP1 as illustrated in Figure 6, page 3236 (see also p. 3233, Col. 2, the paragraph before "Discussion"). The production and cross-reactivity of Abs to the described peptides have been measured in a direct ELISA format. In general, after two injections, a good IgG response was found against the two peptides. Antibodies (Abs) induced against the retro-inverso analogues cross-reacted equally well and even significantly better with the parent, wild-type peptides (Fig. 6B and 6D) than with retro-inverso analogues.

16. In the same way it is shown, in the attached publication (1), S. Muller et al., *Peptide Research*, Vol. 8, No. 3 (1995), 138-144, that the retro-inverso analogues of the two FMDV variants are effective immunogens since they stimulate a long-lasting anti-peptide IgG response and produce antibodies that strongly cross-react with the parent L-peptide (see, Figure 1, page 140 "Cross-reactivity in ELISA of rabbit antisera induced against parent peptide 141-159 of FMDV VP1 and peptide analogues"). Thus, as shown in figure 1C and 1D, antibodies to FP- and FL-R1b analogues reacted equally well with the corresponding R1b, R1a and L-peptides (see also page 142 col. 3). Moreover, the attached publication (2), Briand et al., *Proc. Natl. Acad. Sci. USA*, Vol. 94, pp. 12545-12550, Immunology, shows that antibodies raised against the retro-inverso peptide NH<sub>2</sub>-(C) 141-159-OH cross react strongly with the corresponding L-peptide (see page 12548, col. 2, 1.13).

17. Thus, we believed one of ordinary skill would interpret these additional examples, as demonstrating that they could have made and used the claimed retro-inverso peptide, or retro-peptide, to recognize and bind antibodies raised to parent peptides or proteins or to raise antibodies which will cross-react with the parent, retro-and retro-inverso peptides.

18. The Examiner also asserts

"The disclosure fails to adequately teach which parent peptide-, or protein specific antibodies can reasonably be expected to recognize and bind to retro-inverso peptides, retro peptides or enantiomeric parent peptide." See, ¶(6), page 3 of Paper No. 26.

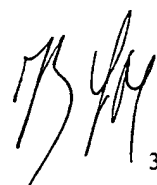


19. Contrary to the Examiner's assertions, we believe one of ordinary skill would have appreciated from our disclosure how to make and use the claimed invention. This is especially true in view of the Examiner's failure to provide any evidence to the contrary.

20. In the attached publication (1), S. Muller et al., *Peptide Research*, Vol. 8, No. 3 (1995), 138-144, it is shown in Figure 1 page 140, that there is cross-reactivity in ELISA of rabbit antisera induced against parent peptide 141-159 of FMDV VP1 and peptide analogues (see also the paragraph "Recognition of peptide analogues by anti-peptide antibodies," page 142, col. 2). Thus, as shown in figure 1A and 1B p. 140, antibodies induced against FP-L- and FL-L-peptides cross-reacted with related RIb (retro-inverso) peptides, although, in two out of three rabbits, with a slightly lower titer.

21. The attached publication (2), Briand et al., *Proc. Natl. Acad. Sci. USA*, Vol. 94, pp. 12545-12550, *Immunology*, shows (see page 12547 colonne 2, "Antigenic mimicry of the region 141-159 of VP1 with retro-inverso peptide analogues" and Fig. 3 page 12548), the capacity of guinea pig antisera raised against intact virus particles, VP1, and L-peptide 141-159 to recognize the retro-inverso peptides 141-159. As shown in Fig. 3A, whatever mode of peptide presentation was used to produce anti-peptide antibodies, the different peptide antisera reacted equally well or better with the retro-inverso analogue NH<sub>2</sub>-(C) 141-159-OH than with L-peptides H-(C)141-159-OH and Ac-(C) 141-159-OH. Similarly, anti-VP1 and anti-virion antisera reacted strongly with both the retro-inverso and L-peptides 141-159.

22. The attached publication (4), Briand et al., *Journal of Biol. Chem.*, Vol. 270, No. 35, 1995, 20686-20691), shows that retro-inverso peptides are recognized as well as or even better





than natural peptides by antibodies from autoimmune patients and lupus mice. See, the whole publication, and, more particularly:

page 20687, col. 2, "Recognition of the retro-inverso analogue of H3 COOH-terminal hexapeptide by sera from lupus mice ... As shown in table II, sera from lupus mice reacted equally well with the L- and the retro-inverso peptides";

page 20687, col. 2, " Recognition of the retro-inverso analogue of Ro 52 277-291 peptide by sera from autoimmune patients";

page 20688, col. 2, "Recognition of the retro-inverso peptide analogue of Ro 60 304-324 peptide by sera from autoimmune patients"; and

page 20689, col. 1, "Recognition of the retro-inverso peptide analogue of H3 28-45 peptide".

These four parent peptides have been already cited in the present patent application and the results are confirmed in the attached publication (4).

23. The attached publication (5), Bradford A. Jameson et al., Nature, Vol. 368, 21.04.1994, 744-746, also shows that the anti-peptide L antibodies recognized as well as or even better, the retro-inverso analogue. Figure 1a (page 744) of this publication is a schematic representation of the backbone structure of the CDR3 region of mouse CD4 receptor and its structurally modified analogues; mPGPtide is the cyclic parent peptide and corresponds to the sequence L3T4 CDR3-like region with a synthetic bridge incorporating a Pro-Gly-Pro motif (PGP). More particularly, the murine peptide proline-glycine-proline "mPGPtide" represents the cyclic structure of the linear m86-104 C-C peptide. "rD-mPGPtide" is the reverse D-mPGPtide

(retro-inverso peptide). Page 744 col. 2 of publication (5) details that "To obtain an immunogenic response from the peptide, a BSA-conjugated version of the L-mPGPtide was repeatedly inoculated into rabbits in the presence of Freund's complete adjuvant. The antisera strongly cross-reacted (at dilutions > 1:10 000) with the m86-104 C-C peptide and both the L- and D-versions of the mPGPtide (Fig. 1b)." Figure 1b (page 744) shows that anti-peptide reactivity in ELISA with mPGPtide (diagonal bars) and m86-104 C-C (horizontal bars) is substantially the same as with rD-mPGPtide (last column of figure 1b).

24. In the same way, the attached publication (6), A. Verdoliva et al., J. Biol. Chem., 270, 1995, 30422-30427, shows that the anti-peptide L antibodies recognized as well as or even better the retro-inverso and retro analogues. See, the abstract page 30422 ("Rabbit polyclonal antibodies against multimeric peptide antigens were found to cross-react to a significant extent with topologically related variants of the parent antigen, where the chirality of each amino acid residue (inverso derivatives), or the peptide sequence orientation (retro derivatives), was inverted or where both modifications were simultaneously introduced (retro-inverso derivatives). All peptides variants displayed similar recognition properties for antibodies..."). The sequences of the peptides used in this study are shown in Figure 1, page 30424 (see, MAP-P15, normal-P15, inverso-P15, retro-P15 and retro-inverso-P15). Scrambled P15 represents a peptide which contains the same amino-acids as the normal-P15 but in a random order. An interesting result is given in figure 2, page 20424, which corresponds to a competition test between anti MAP-P15 antibodies and immobilized MAP-P15, respectively, by peptide P15, retro-P15, inverso-P15, retro-inverso-P15 and scrambled P15. See, more particularly, page 30424, col. 1, which states



"Cross-recognition between the peptide variants was further confirmed by competition experiment microtiter plates coated with the parent antigen MAP-P15. As shown in Fig. 2, all the peptide variants conjugated to BSA were efficiently able to displace the interaction between anti-MAP-P15 antibodies and MAP-P15 immobilized on microtiter plates in a dose-dependent manner, and interaction was reduced at 50% of its original value by BSA-peptide conjugates at roughly the same concentration (Table I). Scrambled linear antigen, on the other hand, had no effect on the interaction. ..."

25. The Examiner asserts that

"Applicants' claimed invention encompasses an enormous number of sundry peptides with unknown biological and immunological activities. Thus, the skilled artisan has only been extended an undue invitation to further experimentation to ascertain which peptides will function in the desired manner."  
See, ¶(7), page 3 of Paper No. 26.

26. As noted above, we do not believe the Examiner has provided any evidence that one of ordinary skill would not be able to make and use the claimed invention. We have, however, provided numerous examples where ordinarily skilled artisans have made and used retro- and retro-inverso peptides, without undue experimentation. Moreover, we note that the analogues 304-324 peptide of Ro52, 277-291 peptide of Ro60, 130-135 of histone H<sub>3</sub> and 28-45 of histone H<sub>3</sub> are recognized by patients' antibodies. We also submit the attached additional publications (7), (8) and (9) which are further summarized below.

27. The article (7) "Retroenantiomers-a close match indeed," July 14, 1997, C&EN, 56-57, and the publication (8) "Solution Structures of FcεRI α-Chain Mimics: A β-hairpin Peptide



and Its Retroenantiomer", McDonnell et al., J. Am. Chem. Soc., 1997, 119, 5321-328 show that retro- or retro-inverso- enantiomers of L-peptides having hairpin structures are topologically or topochemically similar to those of the parent peptides. Thus, in the article (7) "Retroenantiomers-a close match indeed ...", it is said that "Researchers have obtained direct evidence of the expected similarity between the surfaces of a complex cyclic peptide and its retroenantiomer. And they have shown that even short peptides can be designed to form stable  $\beta$ -hairpin structures. ... Confirmation of the topological similarity of retroenantiomeric peptides "has extremely interesting implications for protein design theory and may be of great practical value in the design of drugs that resist proteolysis". The publication (8), McDonnell et al., J. Am. Chem. Soc., 1997, 119, 5321-5328, describes the structure of two retroenantiomeric,  $\beta$ -hairpin-forming-peptide mimics of the high-affinity receptor for IgE. A  $\beta$ -hairpin region comprised of the C-C' strands of the second extracellular domain of the  $\alpha$ -chain of Fc $\epsilon$ RI has been shown to be important in its interaction with IgE. In this work, a set of cyclic peptides designed to mimic this C-C'  $\beta$ -hairpin structure found in the Fc $\epsilon$ RI  $\alpha$ -chain has been synthesized. The cyclo(L-262) and cyclo(rD-262) peptides exhibit a structure similar to that predicted for the C-C' region in the homology-based model of the Fc $\epsilon$ RI  $\alpha$ -chain. Figure 6, page 5327, shows a GRASP surface representation of two similar conformers of cyclo(L-262) and cyclo(rD-262). This topological similarity between the two compounds is reflected in their similar affinity for IgE.


28. The attached publication (9) " Structural Limitations to antigenic mimicry achievable with retro-inverso (all-D-retro) Peptides" Guichard et al., Tibtech, February 1996, Vol. 14, 44-

45, reports on the synthesis of peptide analogues of the immunodominant epitope of the VP1 protein of two variants of the foot-and-mouth disease virus (FMDV), and the demonstration of induction of longer-lasting and higher antibody titers in immunized animals than with the corresponding L-peptide. These peptides appear to be random coils in water solution, and only adopt a partial  $\alpha$ -helical conformation in trifluoroethanol (TFE). Then, in a recent NMR study of a retro-inverso (all-D-retro) FMDV peptide in TFE, the authors have shown that it contained a left-handed helical region, this contrasted with the right-handed helix observed in the native L-peptide form.

29. The attached publication (10) "Recent developments in retro peptides and proteins-an ongoing topochemical exploration." Chorev et al., Tibtech, October 1995, Vol. 13, 438-445, shows that "Antigenicity and immunogenicity can be achieved by metabolically stable antigens such as all-D- and retro-inverso-isomers of natural antigenic peptides". The last paragraph, page 440, relates the works from the present inventors, Muller and Briand; see "The stereochemical enhancement of antigenic mimicry", and more particularly, page 441, "Antibodies of the IgG3 isotype, raised against either the hexapeptide derived from the C-terminus of histone H3 or its enantiomeric all-D-antigen, were able to bind to both enantiomeric antigens. This discovery was followed by the finding that the antibodies did not discriminate among the all-L-, the all-D-, the all-L-retro and the all-D-retro (retro-inverso) isomers. Interestingly, antibodies of the IgG1, IgG2a and IgG2b isotypes distinguish between both pairs of topochemically corresponding isomers, and antibodies raised against the all-L-antigen also recognized its retro-inverso-isomer." Figure 3, page 441, of this publication represents the natural all-L-antigen, its all-D-analogue and



the retro- and retro-inverso- peptides which were already described in the present patent application.

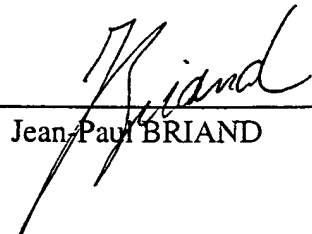
 We hereby declare that all statements made herein of our knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Further, declarants sayeth not.

Signed this 27 day of March, 1999.

  
\_\_\_\_\_  
Syviane MULLER

Signed this 28 day of March, 1999.

  
\_\_\_\_\_  
Jean-Paul BRIAND

